Effect of adrenocorticotrophic hormone on sodium appetite in mice

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ADRENOCORTICOTROPHIC HORMONE (ACTH) has been shown to cause a large specific increase in sodium chloride intake in rabbits (2), sheep (29), and rats (32). In the case of sheep and rats, the effect of ACTH was abolished by adrenalectomy, but with wild rabbits from the Snowy Mountain region of Australia, ACTH had a residual sodium appetite-stimulating effect in animals that had verified adrenalectomy (2). The effect of ACTH was large, involving daily turnover of approximately one-third of the extracellular sodium content of sheep and was equivalent to the total body sodium in rats. The effect of ACTH was reproduced in sheep by intravenous infusion of a cocktail of steroid hormones, which contrived comparable blood levels to those produced by ACTH (8). It has also been shown in mice (3) that adrenal steroids evoked by ACTH will induce sodium appetite. In contrast to the data on these species, it has been found that systemic ACTH given for 5 days does not influence sodium appetite in baboons (R. E. Shade, J. R. Blair-West, and D. A. Denton, unpublished), although increase in blood pressure and blood corticosteroids was observed.

In rabbits, it has been found that intracerebroventricular infusion of ovine corticotrophin releasing factor (CRF) causes an increase in sodium appetite in the first 24 h of administration, and the effect may persist for 2 to 3 days after cessation of administration of the hormone (23). It is accompanied by behavioral disturbances indicative of apprehension and hyperreactivity to stimuli hitherto innocuous.

Overall, the data, coupled with the evidence that stress caused by partial immobilization with jackets will induce a large sodium appetite in rabbits (8, 10) that disappears when the jackets are removed, are indicative that environmental events that threaten the existence and cause stress in an animal can induce a large specific sodium appetite in several, but not all, species as a result of activation of the CRF–ACTH–adrenal steroid cascade. Kuta et al. (17) showed in mice that restraint may cause increase of voluntary sodium intake, an effect that is reduced by the administration of converting enzyme inhibitor (CEI) or naloxone.

The purpose of the experiments reported here was to examine the influence of CRF, ACTH, and stress on the intake of salt and water in mice and to determine also whether the systemic administration of a CEI, which causes a reduction of sodium appetite evoked by sodium deficiency in several species, such as sheep, cattle, rats, and mice (5, 26, 27, 30, 31), also causes reduction of salt or water appetite caused by ACTH.

METHODS

Home-bred BALB/c mice aged 4–9 mo were housed in individual plastic cages (16 × 30 × 12 cm) with sloping grill lids (Wiretainers, Australia). Low-sodium food (Na+: 4–7 mmol/kg; K+: 200–220 mmol/kg; ICN Biochemicals) was available ad libitum on the sloping section of the lid. Water and 0.3 M NaCl solution were offered via 60-ml syringes fitted with glass drinking spouts pushed through the grill lid. When more than one solution was offered, the position of the syringes was changed daily to avoid positional preference drinking. The mouse room was temperature controlled and on a 12:12-h light-dark cycle. Intakes of food and fluids and body weight were measured daily at around 1100.

Surgical Procedures

In experiments involving infusion into the third ventricle of the brain, mice were anesthetized with a mixture of 0.5 ml Ketapex (ketamine 100 mg/ml, Apex Laboratories) and 0.5 ml Rompun (xylazine 20 mg/ml, Bayer Australia) in 9 ml of saline, dose 0.02 ml/g body wt, and then surgically prepared with head probes according to the method described in Denton
et al. in 1990 (9). The solutions were delivered by miniosmotic pumps (ALZA, CA models 2001, 1 µl/h for 7 days, and 2002, 0.5 µl/h for 14 days), attached to the headprobes via Tygon cannulas and placed subcutaneously on the back.

To implant the miniosmotic pumps for the subcutaneous infusions or to exchange them during the intracerebroventricular infusions, the mice were anesthetized with Penthrane (methoxyflurane, Abbott), a short acting inhalation anesthetic. The pump change procedure required ~5 min, and the animals were fully recovered within 10–15 min.

Experimental Procedure

For all studies, intake and body weight were measured daily to establish normal baseline values. The peptides, including ACTH-(1—24) (Synachten or Synachten Depot, Ciba), CRF (ovine CRF, Peninsula), and captopril (Squibb), a CE1, were then infused for 7–10 days followed by a postinfusion period of readings. In the case of intracerebroventricular infusions, both the pre- and postinfusion periods were observed during infusion of artificial cerebrospinal fluid (aCSF). All solutions to be infused intracerebroventricularly were passed through a 0.2 µm filter (Acrodisc, Gelman Scientific).

Statistical Methods

Data are expressed as means ± SE and were analyzed using one- or two-way ANOVA, repeated measures design, and by subsequent comparison of the means using the error mean square of each ANOVA as the estimate of variance.

Experiment 1: Subcutaneous Infusion of ACTH-(1—24) (Synachten) for 7 Days

Six female mice were observed for 5 control days. Miniosmotic pumps dispensing ACTH-(1—24) at 2.8 µg/day were implanted for 7 days. After removal, measurements were made for 18-day postinfusion period. The dosage of ACTH was of the same order on a body weight basis as that found effective on sodium appetite in rabbits (2) or sheep (29), but was increased two- to five-fold in the light of higher basal metabolic rate of mice.

Experiment 2: Intracerebroventricular Infusion of ACTH for 7 Days

Control measurements were made on nine female mice for 5 days during which aCSF was infused. The infusion was changed to intracerebroventricular ACTH-(1—24) (Synachten n = 4 or Synachten Depot n = 5) 20 ng/day for 7 days and then changed back to aCSF for the 4-day postinfusion period. Because there was no difference in the response of the mice to the different forms of ACTH, the resulting data were combined. The dosage was one-thousandth of that which had a large effect systemically and was in the same range as that of intracerebroventricular ANG II (70 ng/day), which caused a massive sodium appetite (9). It corresponded on a body weight basis to that used intracerebroventricularly in sheep and which caused rise in blood pressure and cardiac rate (22).

Experiment 3: Intracerebroventricular and Subcutaneous Infusions of CRF (Ovine)

Two groups of female mice were used for this experiment. Both groups had 7 days of preinfusion baseline readings, a 7-day infusion period of either subcutaneous or intracerebroventricular CRF at 10 ng/h and 2 postinfusion days. The dosage was calculated on a body weight basis relative to that effective with intracerebroventricular infusion in the rabbit (23).

Experiment 4: subcutaneous ACTH with concurrent infusion of captopril. The captopril dosage was based on that previously found effective in mice (17, 27) and on a body weight basis with sheep given intravenous infusion of captopril at 40 mg/h to block water drinking effect of intracerebroventricular infusion of ANG I at 3.8 µg/h (28).

Control measurements were made on six female mice for 7 days preinfusion, and then a miniosmotic pump containing ACTH-(1—24) (Synachten) at 2.8 µg/day was implanted for 4 days and then replaced by a pump containing the same dose of ACTH-(1—24) but with addition of captopril, 2 mg/day, for a further 6 days. At the end of this period the pump was removed, and the mice had a further 6 days of postinfusion readings.

Experiment 4B: subcutaneous ACTH for 10 days. The same six female mice were reused after 14 days recovery. As before, they had 7 days of control readings and were then implanted with a miniosmotic pump delivering ACTH-(1—24) (2.8 µg/day; Synachten) for 10 days. The pump was removed and 6 days of postinfusion readings were taken.

Experiment 5: Induction of High Blood ACTH Concentration by High Dosage of RU-486

Subcutaneous injection of RU-486 (Roussel), 0.3 mg in 0.1 ml, was given daily for 6 days followed by 0.6 mg in 0.1 ml for 3 days in 8 female mice (1). Intakes of food, water, and 0.3 M NaCl and body weight were measured daily. The animals were killed, and blood was taken for ACTH analysis by radioimmunoassay (25).

Experiment 6: Induction of Stress in Mice by Immobilization

Experiment 6B: total immobilization of the torso, but with wheels for running under paws. Twelve male mice were maintained in individual cages for a period of baseline measurements of NaCl solution (0.2 M) and water. Regular high-sodium mouse food was offered (GR2+ Clarke King Australia; Na: ~90 mmol/kg) but not measured. After 8 days of readings, six of the mice were lightly anesthetized with Penthrane and secured to a bent wire support by a jacket of masking tape placed firmly around the torso. This restricted movement but not breathing. The mouse was then suspended over a stainless steel wheel, and the height was adjusted to allow the mouse to stand and run on the wheel, which was very light and free to turn (6). A food pellet as large as possible was placed in a holder within easy reach of the mouse, with the two solutions available on either side of the pellet. The syringe positions were alternated daily and the pellet was refreshed at least twice per day. The mice were maintained under these conditions for a further 8 days. All 12 mice were killed at the end of the experiment, and adrenal, thymus, and body weights were measured.

Experiment 6B: partial immobilization by taping of the torso. The intakes and body weights of five male BALB/c mice were measured for a 7-day control period and then they were lightly anesthetized with Penthrane and their torsos wrapped with 2-cm wide Micropore tape. Their jackets were firm enough to limit flexibility, but not tight enough to inhibit breathing, and were left in place for 7 days. Another light dose of Penthrane anesthesia was required to remove the jackets, and readings continued for 20 days.
**RESULTS**

**Experiment 1**

Subcutaneous infusion of ACTH-(1–24) at 2.8 µg/day caused a large rise in both 0.3 M NaCl and water intakes. NaCl intake increased steadily from a baseline average of 0.58 ± 0.24 ml/day to 6.84 ± 2.32 ml on day 7 of infusion (P < 0.005) and was significantly different from day 3 of infusion. The intake fell as soon as the infusion was stopped, but did not return consistently to baseline levels until the 9th post infusion day (Fig. 1). The water intake followed a similar pattern. From the baseline mean of 2.71 ± 0.17 ml/day, the intake increased, reaching significance on day 3 (P < 0.05) and continuing to increase until reaching a maximum on day 6 (8.06 ± 1.68 ml; P < 0.005). Unlike NaCl intake, the water intake returned to baseline level by day 2 postinfusion. The low-sodium food intake was also elevated significantly from day 2 to 6 of infusion by 1 g/day. Body weight was largely unaffected by the infusion [but day 3, 21.92 ± 0.56 g, was significantly lower (P < 0.05) than the baseline average 22.73 ± 0.64 g]. The body weight stayed low for most of the next 9 postinfusion days and then began to increase again.

**Experiment 2**

The intracerebroventricular infusion of 20 ng/day of ACTH-(1–24) caused no significant changes from the preinfusion intakes of water, 0.3 M NaCl, and low-sodium food or body weight (Fig. 1).

**Experiment 3**

The effects of infusing the same dose of CRF (10 ng/h) via different routes were significantly different for the two groups of mice. The subcutaneous administration of CRF had no effect on water and 0.3 M NaCl intake. Intracerebroventricular infusion, however, produced a steady increase in NaCl intake from a baseline of 0.62 ± 0.13 ml to a maximum of 2.47 ± 0.59 ml on day 6 of infusion (P < 0.05); day 7 was also significantly greater than control. Both days 6 and 7 of intracerebroventricular infusion were significantly different from the equivalent days of subcutaneous infusion (Fig. 2).

Unlike the subcutaneous infusion, intracerebroventricular infusion caused a significant decrease in water intake for the first 2 days of infusion (P < 0.05 vs. intracerebroventricular control and vs. days 1 and 2 of subcutaneous CRF infusion). Water intake then began to increase and was significantly elevated from intracerebroventricular control average on the last 3 days of infusion. These 3 days were again significantly different (P < 0.05) from days 5, 6, and 7 of subcutaneous infusion where intake was relatively steady.

Intake of low-sodium food was significantly different between the two groups at every point during the infusion, except for day 3 when the intakes were almost identical. For the first 2 days the intake during subcutaneous infusion was higher than the intracerebroventricular group, with day 2 subcutaneous intake (4.82 ± 0.17 g) being significantly higher than the subcerebroventricular control average 3.47 ± 0.08 g (P < 0.05). The intake with intracerebroventricular infusion began to increase and was equivalent to the subcutaneous infusion group intake on day 3 and then greater than it for days 4–7. The postinfusion period days did not differ between the groups.

Body weight was unaffected by the subcutaneous infusion of CRF. The body weights of the intracerebroventricular group were significantly lower than the subcutaneous infusion group for days 1–3 of infusion.

**Experiment 4**

The same six mice were used in both experiments, therefore the results could be directly compared. There was no significant difference between the subcutaneous ACTH with CEI or subcutaneous ACTH alone (Fig. 3). Both treatments caused increased 0.3 M NaCl intake, which was significant by day 5 of infusion. The ACTH + CEI treatment did take longer to return to baseline levels when the infusion was stopped. The water intake
was similar. The ACTH treatment (experiment 4B) caused significantly increased intake by day 5 of infusion, whereas ACTH alone (experiment 4A) was not significantly increased until day 7 of infusion. Both groups returned to baseline levels by day 2 postinfusion. Low-sodium food intake was unaffected by either treatment. Body weights were decreased by day 2 (ACTH + CEI) and day 3 (ACTH alone). However, the ACTH alone did not cause much further decrease and returned to the preinfusion levels. The ACTH + CEI treatment continued to decrease body weight throughout the infusion, and it did not recover to baseline levels during postinfusion observations.

Experiment 5

The administration of RU-486 at 0.3 mg/day for 6 days and 0.6 mg/day for 3 days caused no significant change in sodium water or food intake. The plasma ACTH concentration after 10 days of administration was 787 ± 244 pg/ml compared with the control level in unstressed mice of ~100 pg/ml.

Experiment 6A: Immobilization on Running Wheels

The 8-day baseline intakes of 0.2 M NaCl (control 0.48 ± 0.09 ml, experimental 0.66 ± 0.10 ml), and water (control 4.21 ± 0.07 ml, experimental 4.01 ± 0.09 ml) were the same for the two groups of mice. The control group continued unchanged for the duration of the readings (Fig. 4).

The experimental group, however, displayed marked changes in their intakes. The first day on the wheel was characterized by a low intake of water (0.19 ± 0.07 ml, P < 0.005 vs. baseline average) and NaCl (0.15 ± 0.02, not significantly different). The NaCl intake was significantly greater than baseline on days 2 and 3 (P < 0.005) and days 5 and 7 (P < 0.05) and was significantly different from the control group on days 2, 3, 5, 6, and 7.
The experimental group’s water intake remained significantly below baseline average for days 1 and 2 (P < 0.005) and days 3, 6, and 7 (P < 0.05) and was significantly less than the control group’s intake on days 1, 2, and 6 (P < 0.005; Fig. 4).

Comparisons of adrenal gland, thymus, and body weights at post mortem showed some significant changes (Fig. 5). Thymus weights for the control group (36 ± 1 mg) were significantly greater than those of the experimental group with 8 days on wheels (12.8 ± 2.4 mg, P < 0.005), as were body weights (control group 28.40 ± 0.72 g and experimental group 23.38 ± 0.62 g; P < 0.005). In contrast to the thymus, there was, however, no measurable difference in adrenal gland weight relative to body weight.

Experiment 6B

Wrapping the torsos of five male mice had no significant effect on their intakes of 0.3 M NaCl solution or low-sodium food, nor did it effect water intake during the experimental period. When the jackets were removed, however, the water intake increased rapidly and significantly from the control average (2.67 ±
0.16 ml) for most of the 20-day postrestraint period, reaching a maximum of 6.22 ± 0.71 ml on day 6 (Fig. 6). Body weight decreased during the restraint period, but was not significantly lower until the 4th, 6th, and 7th days of restraint. Similar to food intake, body weight stayed below the control average until the 11th day postrestraint, when it then returned to control levels (Fig. 6).

**DISCUSSION**

The results of this study show that ACTH in the blood is a powerful stimulus of sodium appetite in mice, as with other species (2, 8). Intake induced during 24 h exceeded the total sodium content of the body of the mouse. Intracerebroventricular infusion of ACTH had no effect. The vector of effect of ACTH is the steroid secretion evoked by the adrenal cortex. Baseline intakes of mice show no significant difference between sexes, and response in males has been shown to be similar to that in females, as reported here (unpublished). Important loci of action of adrenal steroids in the genesis of sodium appetite have been reported to be in the central nuclei of the amygdala and the bed nucleus of the stria terminalis (21).

It has been shown (4) that ACTH has a direct effect in stimulating thirst in mice, insofar as animals on a low-sodium diet, with no access to sodium solution and thus restricted in ability to increase sodium intake, nonetheless had a very large increment in water intake with ACTH. This is probably a consequence of a redistribution of electrolytes, which causes a rise of plasma Na⁺ concentration (4). Probably this mechanism, as well as the effect of increased sodium intake, influenced water intake here.

The experiments with RU-486 related to the interesting observation, as yet unexplained, that ACTH causes increased salt appetite in adrenalectomized wild rabbits captured in the Snowy Mountains of Australia (8). These animals are subjected to extreme stress of an environment with low Na content of vegetation and cold.

Adrenalectomy abolishes the salt appetite effect of ACTH in sheep and rats (8). Rowland and Fregly (20) reported adrenalectomy did not increase voluntary sodium intake of CD1 mice. Also they found that in adrenalectomized animals immunoreactive aldosterone-like material was present in blood and did not alter with change from high- to low-sodium food. Five days on low-sodium food did not change the animals’ condition. RU-486 in high doses is an antagonist not only to the progesterone receptors but also to corticosterone receptors (1, 12). Lack of feedback from blood corticosterone causes a large rise in ACTH and that result was seen here. However, no change of sodium appetite occurred, indicative of absence of extra adrenal action of ACTH on sodium appetite.

Similar to the large effect on salt appetite produced by thoracic jackets in the wild rabbit (10), immobilization on a running wheel induced salt appetite in mice. The large decrease of thymus weight strongly suggests increased corticosteroid secretion. In the mice the less stressful procedure of wrapping the thorax in plastic tape without impediment to respiration and without immobilization did not have any comparable effect on sodium appetite.

Systemic administration of captoprill contemporaneously with ACTH did not influence the salt appetite and water intake response to systemic ACTH. It could be inferred that the peripheral level of ANG II had no influence on either the production of steroids by the adrenal in response to ACTH or the action of those steroids in the brain to generate sodium appetite. The failure of captoprill to effect sodium appetite caused by deoxycorticosterone acetate in rats has been reported (11), including the failure with intracerebroventricular captoprill (33). The dosage of captoprill used was based on that effective on intravenous infusion in sheep (28) and rat (11). A future experiment of interest would be to test the effect of intracerebroventricular losartan. However, the result does not necessarily contradict that of Kuta et al. (17), in relation to their finding that captoprill reduces sodium appetite generated by stress. Stress may activate the renin-angiotensin system (7, 22).
16, 35). Thus inhibition of that activation by CEI may be involved in the result of Kuta et al (17), as distinct from there being any compromise of the effect of ACTH in stimulating steroid secretion.

Restraint of an animal to cause stress may initiate a hormone cascade beginning with CRF release. It was noteworthy that the intracerebroventricular infusion of CRF after 4–5 days caused an increase in sodium appetite and water intake. However, this effect, although significant, was not large and much less than the effect of ACTH. The results may be related to dosage, but the result suggests that CRF alone did not have a major effect on ACTH release. It would be interesting to examine the effects of CRF concurrent with intracerebroventricular infusion of vasopressin on the stimulation of sodium appetite. Intracerebroventricular CRF infusion caused short-term reduction of food intake and body weight, whereas systemic ACTH caused an increase in food intake. The decrease in food intake with CRF is consistent with findings reported by a number of workers (15, 19).

It is possible that the action of intracerebroventricular CRF in stimulating sodium appetite involves a direct action within the brain on neuronal systems subserving sodium appetite analogous to food intake rather than, or as well as, an action via ACTH release with consequent secretion of steroids that act on the amygdala and bed nucleus of the stria terminalis to cause sodium appetite. It also has to be considered that high levels of intracerebroventricular CRF can increase blood pressure in sheep (22) and rabbits (24) and that a natriuresis could have preceded increase in sodium appetite. However, increase of sodium appetite with intracerebroventricular CRF, which occurred in the first 24 h in rabbits, was sustained over 3 days after infusion ceased (23) and did not result from an antecedent natriuresis.

Perspectives

Data have been accrued in many species indicative that either stress or the components of the hormone cascade evoked by stress have a large effect on salt appetite. This could reflect an important survival advantage in capacity to adapt to stringent environmental circumstances as, for example, with rabbits living in alpine areas with low Na content of vegetation, extreme cold, and the need to sequestrate relatively large amounts of sodium during pregnancy and lactation because they have several litters in a breeding season and 4–8 offspring at a time. Mice also have large litters and inhabit sodium-impoverished environments. A key unresolved issue is whether stress and the hormone cascade generates sodium appetite in humans (34). This is not yet resolved, but has an important implication in human medicine in the light of the possible role of excess salt intake in the genesis of high blood pressure (13, 14, 18).

This work was supported by a grant from the Robert J. Kleberg, Jr., and Helen C. Kleberg Foundation, the Harold G. and Lelia Y. Mathers Charitable Foundation, and the National Health and Medical Research Council of Australia.

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Received 2 November 1998; accepted in final form 7 June 1999.

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